tion of anticonvulsant activity. The more favorable anticonvulsant effects were observed when the phenyl ring of phenobarbital was chlorinated in the 3,4 positions.

Preliminary clinical trial (9) suggested that dichloro in a dose of 300 mg. per day has no significant anticonvulsant activity in man. This was unexpected since the results presented herein for mice and those reported by Gibson, *et al.* (8), for rats indicate that, except for its temporal properties, dichloro has a profile of anticonvulsant action remarkably similar to that of phenobarbital. In view of this dichotomy and because of the well-known usefulness of phenobarbital in epilepsy, the clinical value of dichloro should be unequivocally determined in order to test the validity of current laboratory procedures for screening potentially useful antiepileptic drugs.

SUMMARY

The anticonvulsant potencies (ED_{50}'s) of two experimental chlorinated barbiturates, 5-(4-chlorophenyl)-5-ethylbarbituric acid (monochloro) and 5-(3,4-dichlorophenyl)-5-ethylbarbituric acid (dichloro), and for phenobarbital were determined in mice by the following two tests: maximal electroshock seizure pattern (MES) test and pentylenetetrazol (Metrazol) seizure threshold (s.c. Met.) test. In addition, the dose of each drug fatal to 50% of animals (LD_{50}) and the dose which induced minimal evidence of neurotoxicity in 50% of animals (TD_{50}) were determined. Protective indices (P.I. = TD_{50}/ED_{50}) were calculated. On the basis of the results obtained the following conclusions appear to be justified.

1. Chlorination of phenobarbital modifies the lethal dose independently of the minimal neurotoxic dose.

2. Chlorination extends approximately eightfold the time required for the drug to exhibit peak anticonvulsant activity and prolongs three- to sevenfold the time for anticonvulsant activity to fall 50%.

3. The chlorinated barbiturates exhibit anticonvulsant activity by both tests; *monochloro* is less potent than phenobarbital, whereas the anticonvulsant potency of dichloro is not significantly different from that of phenobarbital.

4. On the basis of P.I.'s, dichloro exhibits the most favorable indices, but the P.I.'s for dichloro are not significantly different from those for phenobarbital. The P.I.'s for all three compounds are higher by the s.c. Met. test than by the MES test, but this difference is significant only in the case of monochloro.

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Structural Studies on the Triterpene Obliquol

By LEMONT B. KIER and WALLACE S. BREY, JR.

Oxidation studies have indicated that the two hydroxyl groups of obliquol are secondary, one of which exhibits some hindrance as in the Oppenauer oxidation. Nuclear magnetic resonance studies have supported the chemical evidence that the second hydroxyl group is in the 12 position in obliquol. The C-18 methyl peak of obliquol diacetate has shifted downfield from the corresponding peak in lanosterol acetate due to perturbation by the 12-acetyl group. Therefore, it was concluded that obliquol has the structure I. The complete NMR spectra of obliquol, lanosterol, and their acetates are interpreted.

IN A PREVIOUS communication (1) there was reported the isolation of a new triterpene, obliquol (I), from the fungus, *Poria obliqua* (Bres.). At that time, obliquol (I) was shown to be a tetracyclic triterpene with two hydroxyl groups. It was shown to possess two double bonds, one of which was shown to be unreactive and assigned to the 8,9 position. The possibility, based on infrared data, was proposed that one of the hydroxyl groups was primary. This has since been found to be a secondary hydroxyl group. Additional studies on the structure of obliquol comprise the contents of this communication.

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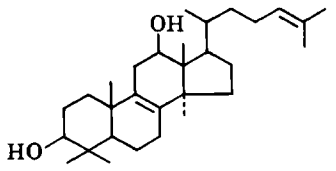
In an effort to elucidate the character of the double bond assumed to be in the side chain, obliquol was subjected to ozonolysis. The isolation of acetone from this treatment proved the presence of the isopropylidene group in the side chain.

Several oxidative procedures were attempted *in order* to locate the hydroxyl groups of obliquol. The Oppenauer oxidation, using various conditions, gave the same compound which, however, could not be thoroughly purified. The infrared spectrum indicated that this compound was an hydroxy ketone. The compound gave a positive Zimmermann test indicating a 3-keto group. The resistance of the second hydroxyl group to Oppenauer oxidation gave the first indication that obliquol contained two secondary hydroxyl groups, one of which was partially hindered.

Subsequent titration of obliquol with a standard acidic chromium trioxide solution confirmed that both hydroxyl groups were secondary since almost exactly two equivalents of oxidant were consumed, indicating the formation of two keto groups by this method.

Oxidation with chromium trioxide in acetic acid gave an impure product which showed no hydroxyl group absorption in the infrared but possessed a strong single band at 1715 cm.$^{-1}$, indicative of one or more keto groups in six-membered rings. This finding eliminated the five-membered D ring as the sight for the second oxygen function (2). Therefore, the positions that this second hydroxyl group could occupy were the 6, 7, 11, or 12. Position 1 could be eliminated since Oppenauer oxidation of a 1,3 dihydroxy system results in the loss of the 1-hydroxyl group (3). A 2-hydroxyl group was eliminated as a possibility since oxidation to an α-diketo system would be detected in the infrared spectrum.

The 7 or 11 hydroxyl group possibilities were eliminated since they would be allylic to the 8,9 double bond, hence quite susceptible to oxidation (4). The 6 α or 6 β hydroxyl groups have also been shown to be susceptible to Oppenauer oxidation (5). This narrowed the possibilities to the two epimeric 12 positions. In the case of the triterpene, polyporenic acid A, the 12 α hydroxyl group is sufficiently hindered to resist benzoylation (6). Obliquol formed a dibenzoate readily, which narrowed the possibilities to the 12 β position for the second hydroxyl group. This position is consistent with the findings but more conclusive evidence was derived from nuclear magnetic resonance data.



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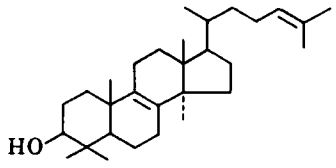

II

The resonances of obliquol (I), lanosterol (II), and their acylated products are discussed in turn, the numbers cited being tau values (see Fig. 1). Starting at low field there appears first the resonances of the olefinic hydrogens on the side chain as a broad triplet between 4.79 and 4.95. The exact value of the shift in obliquol diacetate, where another peak is superimposed on this one (the peak for the H on the same C as the acetoxy group) is in doubt. The H's on the same C as an OH group in the two triterpenes appear next. The C-3 H is at 6.69 and 6.80, while the other H on a C—OH in obliquol falls at 6.29. Acetylation shifts these peaks downfield by about 1.2 p.p.m. In obliquol diacetate they appear at 4.95 (nearly coincident with the olefinic hydrogen peak), and 5.43, and in lanosterol acetate at 5.52. The acetyl methyls appear at 7.85, 7.88, and 8.00. The two terminal methyls appear as a broadened pair of peaks between 8.3 and 8.5 in all compounds except obliquol diacetate. In this derivative there appears at this point in the spectrum a single broad hump with an area which corresponds to three methyl groups. We interpret this as the superposition on the pair of peaks of the isopropylidene methyls with a methyl group of which the chemical shift is reduced by the presence of the second hydroxyl group in obliquol. If this hydroxyl group is at the 12 position, then the nearest methyl group is the 18 methyl group. In corroboration of this, the C-18 peak appears in lanosterol at 9.32 and in lanosterol acetate at 9.35, but in obliquol and obliquol diacetate there are no methyl groups with chemical shifts this high.

The remaining methyl peaks cannot be definitely assigned. One of the peaks, that corresponding to the side chain methyl, should be a doublet. In lanosterol, half of the doublet can be seen at 9.10 and the other half is presumed to

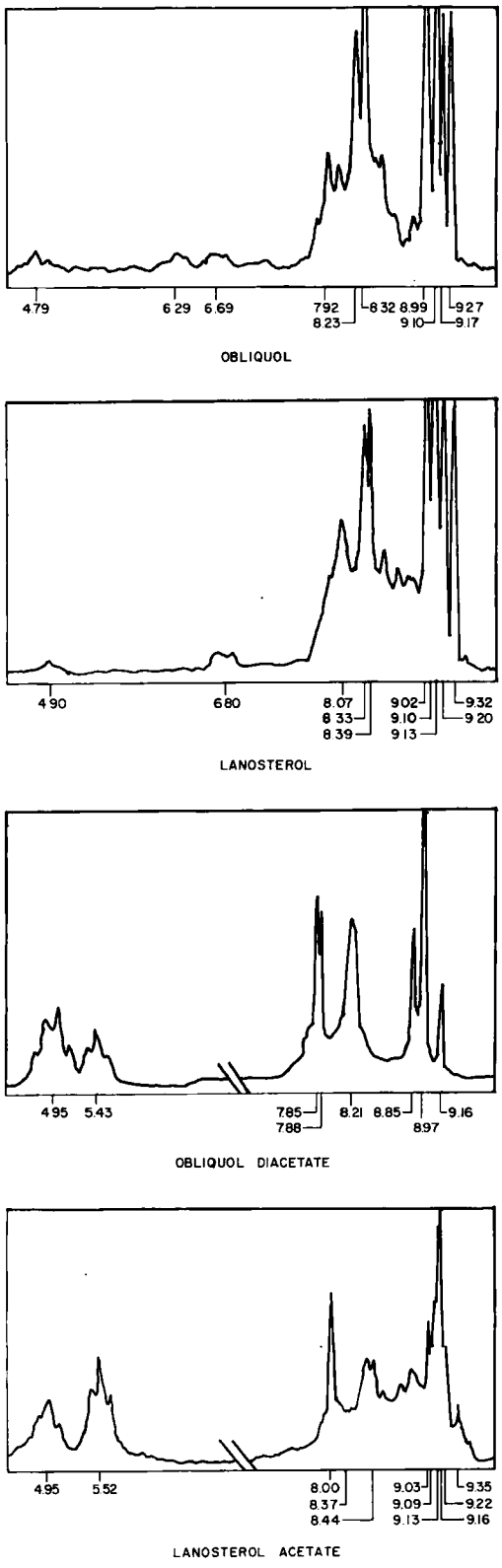

Fig. 1.—Nuclear magnetic resonance spectra of obliquol, lanosterol, and their acetylated products. Numbers are expressed as tau values.

be part of the peak at 9.02, the remainder of this peak corresponding to two other methyls, one of which is probably C-19. The remaining three ring methyl groups, at positions 4 and 14 are at 9.02 and 9.13.

In lanosterol acetate, the C-18 methyl group appears at 9.35, while one other methyl group is clearly defined at 9.03 and the remaining peaks are overlapping in the region surrounding 9.16. The principal effect of acetylation would be expected for the two methyls at position 4, and they seem to have been shifted upfield.

In obliquol, individual methyl peaks appear clearly defined at 9.10, 9.17, and 9.27. Two methyls appear in the resonance at 8.99, one of which is probably C-19. The doublet for the side chain methyl is apparently half in the peak at 8.99 and half in the peak at 9.10. In obliquol diacetate, that which is probably C-19 appears at 8.85, and what is probably the C-18 methyl group appears at 9.16. The remaining methyl groups appear in an unresolvable band at 8.97.

In consideration of the finding of the interference with the C-18 methyl hydrogens by one of the acetyl groups of obliquol diacetate, we conclude that this acetyl group is on the proximate C-12 position. Furthermore, from a study of a model this acetyl group in the 12 position must be β oriented in order to be in a position to interact with the β oriented C-18 methyl hydrogens. This finding was completely consistent with all of the chemical evidence and therefore led us to provisionally assign structure I to obliquol.

EXPERIMENTAL

Nuclear magnetic resonance spectra were determined with a Varian 4300-C spectrometer operating at 56.4 megacycles. The samples were examined as saturated solutions in deuterated chloroform. Chemical shifts were calculated from audio side bands applied by an oscillator with frequency continuously monitored by a Hewlett-Packard 523B counter. The shifts are expressed in tau values computed by using the H of the chloroform in the solvent as an internal reference and adding 2.72 p.p.m. to the shift observed from this reference. Several spectra were also referenced with external benzene, which falls 0.86 p.p.m. to high field of the chloroform peak. In the spectra of the acetates, the regions at low field were run at higher gain than the regions at high field.

Ozonolysis of Obliquol Diacetate.—One hundred milligrams of obliquol diacetate in 20 ml. of carbon tetrachloride was subjected to a stream of 3% ozonized oxygen at −5° for 30 minutes. The solution was then treated with water and powdered zinc and finally steam distilled into an acidic solution of 2,4-dinitrophenylhydrazine. The phenylhydrazone that formed was crystallized, m.p. 122–124°. A mixed melting point with authentic

acetone 2,4-dinitrophenylhydrazone gave no depression.

Oppenauer Oxidation of Obliquol.—A solution of 0.4 Gm. of obliquol in 5 ml. of cyclohexanone and 10 ml. of toluene was treated with 0.8 Gm. of aluminum isopropoxide and refluxed for 2 hours. This solution was cooled and 5 ml. of 10% sulfuric acid added. The solution was then extracted with ether and the residue recrystallized repeatedly from methanol, m.p. 160–165°. Further attempts to purify this compound were unsuccessful. The above procedure was repeated using aluminum tertiary butoxide. It was refluxed for 9 hours. A substance was obtained that was identical to the one obtained by the previous method. It also resisted further purification. Both compounds gave identical infrared spectra. In both cases the compounds gave positive Zimmermann tests but no absorption in the ultraviolet. The infrared spectra showed bands at 3450 cm.$^{-1}$ (O—H), 1715 cm.$^{-1}$ (keto carbonyl), and 1040 cm.$^{-1}$ (C—O).

Chromic Acid Titration of Obliquol.—Obliquol (221 mg., 0.0005 mole) was titrated with a chromium trioxide solution in sulfuric acid that was prepared according to the procedure described by Curtis (7) and Bowers (8). The volume of solution consumed was equivalent to the conversion of 1.95 hydroxyl groups to carbonyl groups.

Chromic Acid–Acetic Acid Oxidation of Obliquol.—A solution of 100 mg. of obliquol in 10 ml. of benzene was treated with 130 mg. of chromium trioxide in 1.5 ml. of water and 3 ml. of glacial acetic acid and allowed to stand for 18 hours. The benzene solution was separated, concentrated, and placed on an alumina column. A small fraction that was eluted with benzene-methanol remained as a viscous oil. The infrared spectrum showed no hydroxyl group absorption but showed a strong carbonyl peak at 1715 cm.$^{-1}$. The substance gave a positive Zimmermann test.

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New Method for Location of Organic Acids on Paper Chromatograms

By GILBERT C. SCHMIDT, CARL FISCHER†, and JOHN M. McOWEN

A modification of Riegler's nitrite test, employing betanaphthol, sodium nitrite, and naphthylamine or sulfanilamide, has been adapted for location of organic acids on paper chromatograms. Most of the twelve acids tested are detectable at a level of 5 mcg. or less although 50–75 mcg. are required in isolated cases. The reagent may be used with all four of the common solvent systems employed, with no notable differences in sensitivity. The sensitivities of this reagent and four previously described detection reagents have been compared. The new reagent is more sensitive than the best of the previously described procedures.

The introduction of paper chromatography as a method for identifying organic acids (1) has been followed by the use of numerous detection reagents. The acid-base indicators have been most frequently used, but colors are transient, complete solvent removal is essential, and it is difficult to distinguish true spots from artefacts. Better results have been reported if the indicator is added to the solvent system (2, 3), but this modification has not been generally accepted. Potassium permanganate, usually used to locate unsaturated and hydroxy compounds (4), has been combined with indicators to produce a general location reagent. The reported sensitivity is poor, but final colors and development times vary for different acids. The combined reagent has therefore been recommended to distinguish acids having similar R_f values (5).

The acid catalyzed condensation of aniline and reducing sugars to form colored compounds has been used to locate both sugars (6) and acids (7). Sugars are detected with an aniline-phosphoric acid mixture, while acids are detected with an aniline-xylose mixture. The aniline-xylose reagent and acridine appear to be the most useful of the remaining detection reagents (7), but both are carcinogenic and little has been written about them.

Nessler's reagent and starch-iodine-iodate reagent have been used to locate the ammonium salts of acids, but neither is a satisfactory general location reagent (7, 8). Aqueous ferric chloride

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